Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Antidiabetic Effects of Aqueous and Dichloromethane/Methanol Stem Bark Extracts of *Pterocarpus soyauxii* Taub (*Papilionaceae*) on Streptozotocin-induced Diabetic Rats

Marie Claire Tchamadeu[1,2,3], Paul Désiré Djomeni Dzeufiet[2], Nelly Blaes[3], Jean-Pierre Girolami[3], Pierre Kamtchouing[2], Théophile Dimo[2]

[1]Department of Animal Biology Organisms, Faculty of Science, University of Douala, 24157, Douala, Cameroon, [2]Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, 812, Yaounde, Cameroon, [3]INSERM, U1048, I2MC Institute of Metabolic and Cardiovascular diseases, University of Toulouse III, F31432, Toulouse, France

ABSTRACT

Aim of the Study: The aim is to evaluate the hypoglycemic and antidiabetic effects of aqueous and CH_2Cl_2/CH_3OH stem bark extracts of *Pterocarpus soyauxii* Taub in normal and diabetic rats. **Materials and Methods:** Streptozotocin (STZ)-induced diabetic and normal adult Wistar rats were orally administered with aqueous and CH_2Cl_2/CH_3OH plant extracts of *P. soyauxii* at various doses (38–300 mg/kg) in a single administration. In addition, STZ-induced diabetic rats received prolonged daily administration for 14 days. Glibenclamide (GB) (10 mg/kg) was used as reference treatment. In acute test, fasting blood glucose was followed for 5 h. In subacute test, body weight, food and water intakes, and blood glucose were followed weekly and serum biochemical parameters evaluated after 14 days treatment. **Results**: Acute administration of aqueous and CH_2Cl_2/CH_3OH stem bark extracts moderately decreased fasting blood glucose compared to GB, significantly in normal rats ($P < 0.05$ to $P < 0.01$) but, as GB, not significantly in diabetic rats. Prolonged treatments in diabetic rats with aqueous and CH_2Cl_2/CH_3OH extracts reduced blood glucose to an extent, respectively, superior or similar to GB. Moreover, *P. soyauxii* also significantly ($P < 0.01$) reduced weight loss, and diabetes increased serum triglycerides, total cholesterol, and transaminases (alanine aminotransferase/aspartate aminotransferase) elevations. **Conclusion:** *P. soyauxii* Taub stem bark extracts have possible value for antidiabetic oral medication.

Key words: Antihyperglycemic, diabetes mellitus, phytotherapy, *Pterocarpus soyauxii* Taub, rats, streptozotocin

SUMMARY

- Aqueous and Dichloromethane/Methanol stem bark extracts of *Pterocarpus soyauxii* Taub have potent (compared to Glibenclamide) antidiabetic effects in STZ-diabetic rats, with specific kinetics and dose-responses.
- Moderate hypoglycemia effects upon acute *P. soyauxii* administration.
- Potent anti-hyperglycemic effects of sub-acute *P. soyauxii* administration in STZ-diabetic rats.
- Potent anti-hyperlipidemic effects of sub-acute *P. soyauxii* administration in STZ-diabetic rats.
- Improved hepatic and renal serum parameters after sub-acute *P. soyauxii* administration in STZ-diabetic rats.
- *P. soyauxii* extracts may be useful for oral treatment of diabetes and related metabolic disorders.

Abbreviations Used: CH2Cl2/CH3OH: Dichloromethane/Methanol; STZ: Streptozotocin; GB: Glibenclamide; AE: Aqueous extract; OE: Organic extract; FeCl3: Iron (III) chloride; NaCl: Sodium chloride; K3Fe(CN)6: Potassium ferricyanide; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; H: Hour; BW: Body weight, W1 and W2: Weeks 1 and 2; CHOD: Cholesterol oxidase; GPO: Glycerol-3 Phosphate oxidase; PAP: Phenol + Aminophenazone

Correspondence:
Dr. Marie Claire Tchamadeu,
Department of Animal Biology Organisms,
Faculty of Science, University of Douala, P. O. Box: 24157, Douala, Cameroon.
E-mail: marieclaire_tchamadeu@yahoo.fr

DOI: 10.4103/0974-8490.199767

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by high glucose level in blood and altered metabolism, is a growing health problem. Diabetes affected 387 million (8.3%) people worldwide in 2014, with an increasing high percentage in poor and developing countries.[1] In Africa (including Cameroon), almost 5% of the population is diabetic and a high number could be undiagnosed.[2] In addition to common Type 1, Type 2, and gestational diabetes, ketosis-prone atypical diabetes is mostly seen in populations from African origin.[3] Type 1 diabetes in Africa may somewhat differ from typical European, with later age at onset. Costs of modern therapies, associated with well-known side effects, orient most patients of poor countries toward traditional medicine.[4]

Validation of alternative therapies is of great interest.[5] Medicinal plants provideexcitingnewtherapeuticopportunities,[6]andanumberofmedicinal plants were reported to show antidiabetic potential.[4] Phytochemicals with antidiabetic properties include saponins,[7] flavonoids,[8] phenolics,[9] and other antioxidant compounds.[10] African medicinal plants are evaluated for their antidiabetic potentials,[11,12] with paucity studied in Cameroon.[13,14] In that context, after an ethnobotanical survey in the central region of Cameroon, we chose to study *Pterocarpus soyauxii* Taub, a medicinal plant used in traditional medicine.

Cite this article as: Tchamadeu MC, Dzeufiet PD, Blaes N, Girolami JP, Kamtchouing P, Dimo T. Antidiabetic effects of aqueous and dichloromethane/methanol stem bark extracts of *Pterocarpus soyauxii* Taub (*Papilionaceae*) on streptozotocin-induced diabetic rats. Phcog Res 2017;9:80-6.

P. soyauxii Taub (*Papilionaceae*) is a deciduous rain forest tree of the genus *Pterocarpus* belonging to the family of *Fabaceae* or *Papilionaceae*, in the branch of spermaphytes. Leaves, wood, stem bark, seeds, and flours are used in African, especially Cameroonian pharmacopeia, to treat various diseases including hypertension, diabetes, gastrointestinal parasites, and renal and cutaneous diseases. Leaves of *P. soyauxii* added to food were reported to normalize hematological alterations associated with diabetes mellitus.[15]

Despite knowledge on medicinal potential of *P. soyauxii,* there is a lack of experimental reports on pharmacological activities, particularly in diabetes mellitus. Besides, hypoglycemic and antidiabetic activities of crude extracts of other species of the genus *Pterocarpus* are largely investigated, i.e. for *Pterocarpus marsupium*[4,16] and *Pterocarpus santalinus*.[17,18] Recently, aqueous *P. soyauxii* plant extracts were shown devoid of toxic effects after oral administration to rodents.[19]

The main goal of the present study was to evaluate *P. soyauxii* for hypoglycemic and antidiabetic activities in normal and diabetic rats. We assessed oral administration of aqueous extraction (noted AE) and dichloromethane/methanol (CH_2Cl_2/CH_3OH) (noted organic extraction [OE]) stem bark extracts of *P. soyauxii* Taub on glycemia, either after acute administration in normal and streptozotocin (STZ)-induced diabetic rats or after subacute (14 days) treatment in STZ-induced diabetic rats. Glibenclamide (GB) was used as reference antidiabetic treatment (positive control).[8] In addition, we investigated subacute impacts on body parameters (weight, food and water intakes) and serum biochemical parameters (total proteins, creatinine, total cholesterol, triglycerides, and alanine aminotransferase/aspartate aminotransferase [ALT/AST] activities).

MATERIALS AND METHODS

Chemicals

GB was obtained from Mylan Laboratory. STZ was purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Blood glucose test strips were Accu-chek PLUS from Roche Diagnostics (Mannheim, Germany). All other reagents and chemicals used in the study were extra pure analytical grade obtained from common commercial suppliers.

Plant material

P. soyauxii (*Papilionaceae*) barks were collected in March 2002 in Nkolbibanda village (Central Region, Cameroon) by Dr. Louis Zapfack (Botany Department, University of Yaounde 1). The plant was identified at the National Herbarium of Yaounde where a voucher specimen was deposited (HNC/2427).[19]

Preparation of aqueous and organic extracts of *Pterocarpus soyauxii*

Stem barks were dried at room temperature and ground into powder. For AE, dry powder (200 g) was macerated in 2 L of boiling distilled water for 10 min and then kept 24 h at room temperature before filtering. The filtrate was concentrated in a drying room at 40°C, yielding 24.10 g (w/w 12%) red well-dried aqueous residue, and stored at −20°C until use as previously reported.[19]

For dichloromethane/methanolic extraction (OE), 2 kg of dried stem bark powder was macerated 24 h at room temperature in 5 L CH_2Cl_2/CH_3OH (1:1) and filtered. The filtrate was concentrated using a rotary evaporator, yielding 459.60 g (w/w 23%) of red dried residue, and stored at room temperature.

For each series of experiments, the AE and OE were weighed and dissolved in distilled water to obtain 30 mg/ml stock solutions. Fixation of plant dosing for administration to rats was based on usual dosage by traditional healer (i.e. around 20 mg/kg dried stem barks/body weight [BW]) and on previous animal studies with *P. marsupium*,[16] *P. santalinus*,[20] and *P. soyauxii*.[19] Plant materials were extracted separately in two solvents (water and CH_2Cl_2/CH_3OH); however, dissolution of the two dried filtrates being well done in water, we only performed water control vehicle.

Phytochemical screening

Qualitative phytochemical screenings of the aqueous (AE) and CH_2Cl_2/CH_3OH (OE) stem bark extracts of *P. soyauxii* were carried out following standard procedures as previously described[21] to reveal the presence of alkaloids (Mayer and Dragendorff's test), tannins ($FeCl_3$ test), saponins (frothing test), lipids (Whatman paper test), flavonoids (Shinoda's test), glycosides and polyoses (NaCl and Fehling's solutions A and B), anthraquinones (ether–ammoniac), phenols ($FeCl_3$ test), polyphenols ($FeCl_3$ and $K_3Fe(CN)_6$ test), and terpenoids (Liebermann–Burchard test), as previously used to screen the aqueous *P. soyauxii* extract.[19]

Animals

Adult male albino Wistar rats (3-month-old weighing 200–250 g) were used. They were raised in the animal core facility of the Faculty of Science, University of Yaoundé 1. They were housed in colony cages (5 rats per cage), at controlled room temperature (23°C ± 0.5°C) and humidity (75% ±5%), on a 12 h light/dark cycle and allowed free access to tap water and standard rat diet. Before testing for blood glucose level, the rats were fasted overnight for 12 h, with free access to water. All animal experiments were conducted in accordance with the International Guidelines for Care and Use of Laboratory Animals as described in the European Community Guidelines (EEC Directive 2010/63/EU of the September 22, 2010).

Induction of diabetes mellitus

Diabetes was induced by a single intravenous injection (caudal vein) of STZ (55 mg/kg freshly prepared in ice cold 0.9% saline solution)[22] in overnight-fasted rats anesthetized by ketamine and xylazine (50 mg/kg, 10 mg/kg, i.p.) to avoid pain and stress.[21] Procedure was performed in darkness to avoid degradation of STZ. Control rats received the vehicle alone. Three days after STZ injection, rats with a fasting blood glucose level of at least 250 mg/dL were considered diabetic and used in the experiments.

Measurement of fasting blood glucose level

Blood drop sample was collected from overnight-fasted rats and determination of blood glucose was carried out by glucose-peroxidase method using test strips (Accu-chek PLUS) and an appropriate glucose meter (Accu-chek, Roche Diagnostics, USA). More precisely, for fasting blood glucose determination (at 0, 1, 2, 3, and 5 h for acute experiment and at 0, 8, and 15 days for subacute experiment), the rat was covered with a clean cloth, the tail tip was slightly injured, and the released blood drop deposited on the reactive zone of a strip connected to the glucometer. Repeated bleeding was feasible in the short term by removing the clot.[23]

Experimental design for evaluating acute effects of *Pterocarpus soyauxii* extracts in normal and diabetic rats

A total of 100 rats were used.

Fifty nondiabetic rats randomly divided into ten groups (five rats each):

- Group 1: Nondiabetic control (NC) rats received distilled water (10 mL/kg)
- Groups 2–5: Nondiabetic rats administered with aqueous *P. soyauxii* extract at different doses (AE 38, 75, 150, and 300 mg/kg, respectively)
- Groups 6–9: Nondiabetic rats administered with dichloromethane/

methanol extract at different doses (OE 38, 75, 150, and 300 mg/kg, respectively)
- Group 10: Nondiabetic positive control rats administered with GB (10 mg/kg).

Moreover, fifty STZ-diabetic rats randomly assigned into ten groups (five rats each):
- Group 11: Diabetic control (DC) rats received distilled water (10 mL/kg)
- Groups 12–15: Diabetic rats administered with the aqueous extract at different doses (AE 38, 75, 150, and 300 mg/kg, respectively)
- Groups 16–19: Diabetic rats administered with the dichloromethane/methanol extract at different doses (OE 38, 75, 150, and 300 mg/kg, respectively)
- Group 20: Diabetic positive control rats administered with GB (10 mg/kg).

All groups of rats received a single oral administration of the treatments by gavage. Blood glucose levels were measured before treatment administration (0 h) and 1, 2, 3, and 5 h after.

Experimental design for evaluating subacute effects of *Pterocarpus soyauxii* extracts in diabetic rats

A total number of 55 rats were used, fifty diabetic rats randomly assigned into ten diabetic groups (5 rats in each) and a group of five nondiabetic rats. Rats were daily treated by gavage for 14 days beginning 3 days after STZ treatment with the respective drug or vehicle as follows:
- Group 1: NC rats received 10 mL/kg of distilled water
- Group 2: DC rats received 10 mL/kg of distilled water
- Groups 3–6: Diabetic rats administered with the AE at different doses (38, 75, 150, and 300 mg/kg, respectively)
- Groups 7–10: Diabetic rats administered with the dichloromethane/methanol extract (OE) at different doses (38, 75, 150, and 300 mg/kg, respectively)
- Group 11: Diabetic positive control rats administered with GB (10 mg/kg).

Blood glucose level was measured in 12 h fasted rats before the first treatment administration (day 0) and weekly (day 8 and day 15, respectively, weeks W1 and W2). Body weight (BW) and food and water intakes were recorded daily. At the end of the experimental period, on day 15 (18 days post-STZ), after fasting blood glucose determination, the rats were anesthetized (ketamine 50 mg/kg and xylazine 10 mg/kg, i.p.)[24] and blood samples were collected from the abdominal aorta accessed via laparotomy.[21] The anesthetized animals were then euthanized by decapitation. The serum obtained after blood centrifugation (3000 g, 10 min) was stored at −20°C until analysis.

Biochemical analysis of serum

The serum was analyzed using commercially available diagnostic kits (Fortress Diagnostics, Antrim, UK) for total proteins (Biuret), creatinine (enzymatic UV), total cholesterol (CHOD-PAP method), triglycerides (GPO-PAP method), ALT (colorimetric), AST (colorimetric).

Statistical analysis

Data are presented as mean ± standard error of mean. One-way analysis of variance with Dunnett's multiple comparison posttest was performed to assess differences between groups (GraphPad Software, San Diego, California, USA). P <0.05 was considered statistically significant.

RESULTS

Phytochemical screening of aqueous and CH_2Cl_2/CH_3OH extracts of *Pterocarpus soyauxii*

According to the previous experiments with the experimental methods used,[19] the screening evidenced alkaloids, saponins, flavonoids, tannins, terpenoids, phenols, polyphenols, and glucids classes; no lipid component was revealed.

Effect of single doses of *Pterocarpus soyauxii* extracts on blood glucose of normal rats

Single administration of aqueous (AE) and organic CH_2Cl_2/CH_3OH (OE) stem bark extracts of *P. soyauxii* and antidiabetic treatment GB (10 mg/kg) produced hypoglycemic effects in nondiabetic rats with time-dependent reduction of glycemia [Figure 1a and b]. GB was the most efficient with a maximum fall in glycemia to 38.8 ± 4.4 mg/dL at 5 h (P < 0.001 compared with NC group or to time 0). The AE induced a fall to 68.2 ± 3.3–72 ± 2.2 mg/dL with the 150–300 mg/kg doses, respectively, (P < 0.001) [Figure 1a] and the OE induced a maximum fall to 78.4 ± 5.1 mg/dL at 150 mg/kg (P < 0.001) [Figure 1b].

Effect of single doses of *Pterocarpus soyauxii* extracts on blood glucose of streptozotocin-diabetic rats

Blood glucose level was increased over 350 mg/dl in diabetic rats, 3 days after induction. Single administration of AE (38–300 mg/kg) did not significantly alter hyperglycemia [Figure 2a]. Administration of OE tended to reduce glycemia similarly to GB [Figure 2b].

Subacute effects of *Pterocarpus soyauxii* extracts on blood glucose level of streptozotocin-diabetic rats

Persistent hyperglycemia was observed in DC rats (glycemia up to 373 mg/dL at day 14). Hyperglycemia was significantly reduced by AE and OE treatments from day 7 [Figure 3a and b, respectively]. The AE dose-dependently reduced glycemia up to complete correction to normal blood glucose value (with the AE 150 and 300 mg/kg doses from day 7, P < 0.001 compared to DC) [Figure 3a]. The OE time-dependently reduced hyperglycemia. Maximum lowering to 142.6 ± 8.4 and 127.4 ± 9.6 mg/dL of blood glucose was observed at day 14 with the OE 38 and 75 mg/kg doses, respectively (P < 0.001 compared to DC) [Figure 3b]. Similarly to OE, GB time-dependently reduced hyperglycemia up to 172.4 ± 46.5 mg/dL (P < 0.001).

Subacute effects of *Pterocarpus soyauxii* extracts on body weight and food and water intake of streptozotocin-diabetic rats

In the NC group, BW increased (positive percent change) during weeks 1 and 2 (W1 and W2) [Table 1]. In untreated diabetic (DC) rats, BW decreased (P < 0.01 compared to NC) whereas food and water consumptions markedly increased. AE treatment reduced BW loss (AE 150 and 300 mg/kg doses; P < 0.05–0.01 compared to DC). These high AE doses also lowered excess intakes (P < 0.01 compared to DC). OE did not reduce BW loss and intakes of food and water. GB failed to reduce BW loss and partly diminished consumptions.

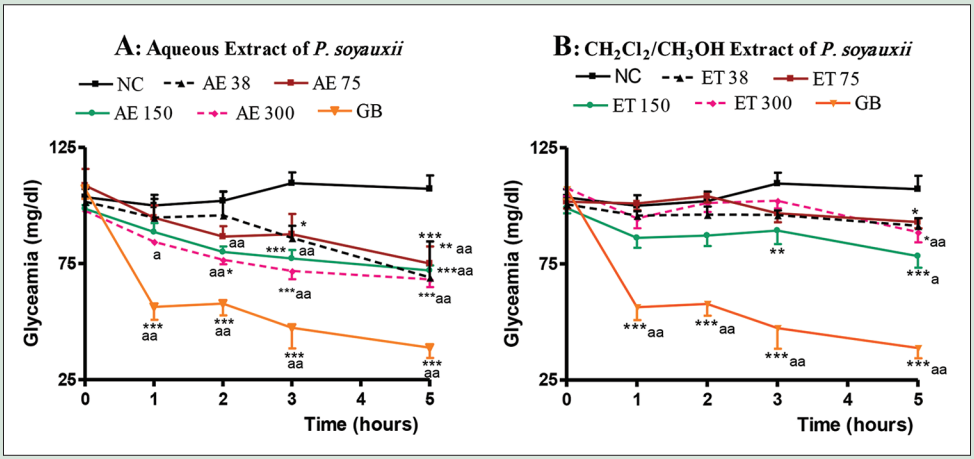

Figure 1: Effects of single doses of aqueous (A) and CH_2Cl_2/CH_3OH (B) *P. soyauxii* extracts on blood glucose level of non diabetic rats. Results are expressed as mean ± SEM, $n = 5$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to NC; $^{a}P < 0.05$; $^{aa}P < 0.01$ compared to initial value (0 h). AE: Aqueous extract at the doses indicated in mg/kg; OE: CH_2Cl_2/CH_3OH extract at the doses indicated in mg/kg; GB: Glibenclamide at 10 mg/kg; NC: Non diabetic control

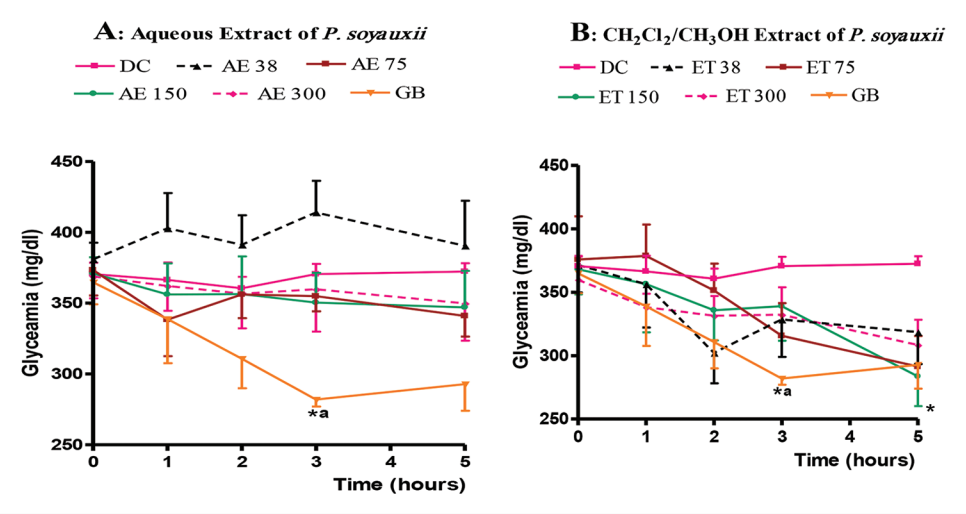

Figure 2: Effects of single doses of aqueous (A) and CH_2Cl_2/CH_3OH (B) *P. soyauxii* extracts on blood glucose level of STZ-diabetic rats. Results are expressed as mean ± SEM, $n = 5$; *$P < 0.05$ compared to diabetic control DC; $^{a}P < 0.05$ compared to initial value (0h). AE: Aqueous extract at the doses indicated; OE: CH_2Cl_2/CH_3OH extract at the doses indicated; GB: Glibenclamide at 10 mg/kg; DC: Diabetic control

Subacute effects of *Pterocarpus soyauxii* extracts on serum biochemical parameters of streptozotocin-diabetic rats

At the end of the experiment, DC rats had significant increases in serum total cholesterol, triglycerides, creatinine, and ALT activity ($P < 0.01$ compared to NC rats) [Table 2]. Trends to decrease in serum total protein and increase in AST were not significant. AE normalized total cholesterol, triglycerides, creatinine (AE 38–300 mg/kg; $P < 0.05$–0.01 compared to DC), and ALT (AE 150–300 mg/kg; $P < 0.01$), lowered AST (AE 38–150 mg/kg; $P < 0.01$), and increased total serum protein (AE 38–75 mg/kg; $P < 0.01$). OE reduced total cholesterol and triglycerides (OE 38–300 and OE 75–150, respectively; $P < 0.05$–0.01), normalized creatinine (OE 38–150 mg/kg; $P < 0.01$), and lowered ALT and AST (OE 38–300; $P < 0.05$–0.01). The high OE 300 dose did not improve creatinine, triglycerides, and total protein. By contrast, GB restored ALT only ($P < 0.01$ compared to DC) but did not significantly change other (total protein, creatinine, total cholesterol, triglycerides) serum parameters.

DISCUSSION

Plants of the *Pterocarpus* genus (*P. marsupium*[4] and *P. santalinus*[18]) have established antidiabetic properties. In the present study, hypoglycemic and antidiabetic effects of aqueous (AE) and CH_2Cl_2/CH_3OH (OE) *P. soyauxii* Taub stem bark extracts were studied against STZ-induced type 1 diabetes mellitus in rats, GB being used as antidiabetic positive control.[8] The STZ model is widely used to test potential antidiabetic properties of natural products derived from medicinal plants.[25] Diabetes in the STZ-induced hyperglycemic rats was confirmed here by BW loss, increased food and water intakes and at the 18th day post STZ injection, by altered serum parameters such as hyperlipidemia (elevated serum triglycerides and total cholesterol), elevated creatinine and ALT activity's, and trends to hypoprotidemia and elevated AST activity's. Herein, the results indicate for the first time that *P. soyauxii* bark extracts induce potent antidiabetic activities. The data enlarge the panel of antidiabetic plants in the *Pterocarpus* genus, with *P. soyauxii* (bark).

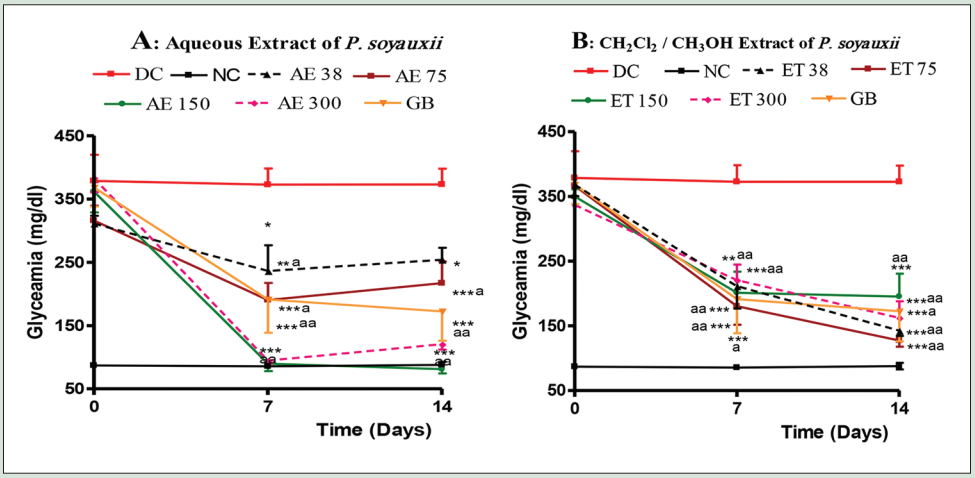

Figure 3: Effects of sub-acute treatment with aqueous (A) and CH_2Cl_2/CH_3OH (B) *P. soyauxii* extracts on blood glucose level of STZ-diabetic rats. Results expressed as mean ± SEM, $n = 5$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to DC; $^{a}P < 0.05$; $^{aa}P < 0.01$ compared to initial value (day 0). AE: Aqueous extract at the doses indicated; OE: CH_2Cl_2/CH_3OH extract at the doses indicated; GB: Glibenclamide at 10 mg/kg; DC: Diabetic control; NC: Non diabetic control

Table 1: Effects of sub-acute treatment with aqueous and CH_2Cl_2/CH_3OH extracts of *P. soyauxii* on body weight, food and water consumptions of diabetic rats

Group treatment	Body Weight change (%/rat/day)		Food Consumption (g/rat/day)		Water Consumption (ml/rat/day)	
	W1	W2	W1	W2	W1	W2
Non diabetic (NC)	+5.09±0.11	+9.44±0.42^{bb}	20.95±0.77	18.46±0.21	19.17±1.30	19.91±1.38
Diabetic (DC)	-11.2±0.51**	-13.69±1.81**	30.51±2.15**	41.62±3.15**^{b}	113.17±0.25**	131.93±8.03**
AE extract						
38 mg/kg	-14.85±2.34**	-26.13±4.16**^{bb}	29.86±4.77	45.71±2.25**^{bb}	111.22±19.00**	129.35±3.96**
75 mg/kg	-14.23±2.45**	-27.23±3.87**^{bb}	38.77±5.32**	44.35±3.38**^{b}	120.27±13.88**	136.5±12.59**
150 mg/kg	-11.44±2.20**	-8.59±2.28*^{a}	31.79±3.43*	26.52±8.84^{aa}	80.83±5.42**^{a}	66.47±5.92**^{aa}
300 mg/kg	-8.18±1.59*	-2.32±0.20^{aa}	36.31±8.82**	29.48±9.58*^{a}	101.09±3.82**^{aa}	91.42±5.68**^{aa}
OE extract						
38 mg/kg	-14.12±4.87**	-18.02±2.27**	44.18±3.43**^{aa}	54.61±9.08**^{ab}	126.90±4.54**	127.75±7.50**
75 mg/kg	-14.40±1.86**	-16.36±2.67**	35.12±8.29**	46.34±5.18**^{bb}	112.36±8.38**	109.30±11.17**
150 mg/kg	-17.42±1.71**	-20.17±1.28**	32.03±1.88**	26.94±3.59**	100.56±1.00**	98.70±3.39**^{a}
300 mg/kg	-12.52±0.90**	-13.58±2.06**	29.81±3.88*	35.22±0.77**	113.10±1.77**	115.03±7.63**
GB (10 mg/kg)	-12.66±1.05**	-16.18±1.64**	18.45±4.24^{a}	29.27±5.58*^{ab}	63.28±0.10*^{aa}	103.40±8.92**^{aabb}

Results are expressed as mean±SEM, n=5; $^{*}P$<0.05, $^{**}P$<0,01 compared to NC; ^{a}P<0.05; ^{aa}P<0.01 compared to DC; ^{b}P<0.05, ^{bb}P<0.01 compared to W1. The weight of the animals is expressed as percent change of the average body weight (BW) per week compared to the initial BW; DC: Diabetic control; OE: CH_2Cl_2/CH_3OH extract; GB: Glibenclamide; NC: Non diabetic control; W1 or W2: Week 1 or 2

Table 2: Effects of sub-acute treatment with aqueous and CH_2Cl_2/CH_3OH *P. soyauxii* extracts on serum biochemical parameters of diabetic rats

Group Treatment	Total Protein (g/L)	Creatinine (mg/L)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	ALT (U/L)	AST (U/L)
Non diabetic (NC)	92.93±3.25	4.25±0.23	71.56±7.62	65.96±6.62	68.65±7.87	57.20±1.00
Diabetic (DC)	73.32±0.35^{ns}	6.51±0,13**	150.30±7.38**	154.56±1.96**	126.32±4.87**	77.40±4.52^{ns}
AE Extract						
38 mg/kg	121.4±14.98^{aa}	3.27±0.23^{aa}	46.19±13.82^{aa}	80.60±3.76aa	100.82±4.08^{ns}	33.04±1.20*^{aa}
75 mg/kg	117.28±11.52^{aa}	3.36±0.34^{aa}	72.04±9.95^{aa}	107.37±11.28*^{a}	107.76±9.18^{ns}	34.40±5.40*^{aa}
150 mg/kg	90.61±0.08^{ns}	3.09±0.71^{aa}	72.99±2.88^{aa}	42.30±7.38^{aa}	38.56±2.86^{aa}	51.42±3.81^{aa}
300 mg/kg	81.25±5.50^{ns}	3.19±0.24^{aa}	65.06±13.22^{aa}	30.56±2.46^{aa}	46.46±6.67^{aa}	73.70±4.08^{ns}
OE Extract						
38 mg/kg	107.14±1.15^{a}	3.27±1.14^{aa}	102.60±3.32^{a}	150.67±7.97**	64.69±8.67^{aa}	29.68±4.80**^{aa}
75 mg/kg	89.17±9.22^{ns}	3.45±0.91^{aa}	91.07±11.05^{aa}	92.10±0.05^{aa}	54.90±4.59^{aa}	21.36±2.40**^{aa}
150 mg/kg	66.55±11.70^{ns}	3.28±0.47^{aa}	64.83±10.35^{aa}	48.86±13.94^{aa}	30.26±1.83^{aa}	67.11±5.01^{ns}
300 mg/kg	133.64±11.52*^{aa}	5.23±1.08^{ns}	76.05±2.16^{aa}	118.37±8.77**	82.14±0.77^{a}	39.56±1.80^{aa}
GB (10 mg/kg)	59.58±3.70^{ns}	6.15±0.33*	128.19±10.29**	128.20±10.29**	36.56±3.60^{aa}	59.80±2.00^{ns}

Results expressed as mean±SEM, n=5; $^{*}P$<0.05; $^{**}P$<0.01 compared to NC. ^{a}P<0.05; ^{aa}P<0.01 compared to DC; ns: non-significant. DC: diabetic control; OE: CH_2Cl_2/CH_3OH extract; GB: Glibenclamide; NC: Non diabetic control

In acute administration, both the aqueous (AE) and CH_2Cl_2/CH_3OH (OE) stem bark extracts of *P. soyauxii* decreased blood glucose level, moderately compared to GB, and significantly in normal rats but nonsignificantly in diabetic rats. Interestingly, in the *Pterocarpus* genus, *P. marsupium*[26] demonstrated hypoglycemic activity in normal rats whereas *P. santalinus* did not but reduce glycemia in diabetic rats.[17]

GB is known to stimulate insulin from remnant β-cells and to inhibit glucagon secretion.[8] Since severe hypoglycemia due to excess insulin secretion is a major life-threatening limitation to pharmacological diabetes treatment, the low hypoglycemiant effect of *P. soyauxii* may be a desirable feature.

The most important result of the present study was the observation that a 14-day treatment with *P. soyauxii* extracts reduced hyperglycemia in diabetic rats at a magnitude superior to GB. OE at low doses (38–75 mg/kg) was slightly more efficient than GB and showed similar kinetics. Besides, AE (150–300 mg/kg) completely normalized glycemia from the treatment day 7. For the *Pterocarpus* genus, the ethylacetate: Methanol (9:1) fraction of *P. santalinus* bark ethanolic (150 mg/kg/day) extract[20] and aqueous or methanolic *P. marsupium* extracts (at 0.5–1 g/kg/day[26] or 150 mg/kg/day[16]) showed antihyperglycemic activity, but without recovery of normal glycemia. Therefore, *P. soyauxii* evidenced marked antihyperglycemic effects, in which potency could be further compared to other *Pterocarpus* species and plants.[4,11]

Type 1 diabetes is associated to alterations of general body parameters, weight loss, polydipsia, and polyphagia. BW loss, despite increased food intake, is known to be due to insulin deficiency induced catabolism of proteins and muscle wasting. Consistent with its potent antihyperglycemic effect, the aqueous *P. soyauxii* extract (150 and 300 mg/kg) reduced these general alterations and much better than OE extracts and GB. Reduction in BW loss with *P. soyauxii* AE extracts argues for improved insulin secretion as observed for *P. santalinus*.[20]

Inadequate protein catabolism with increased serum creatinine may cause glomerular dysfunction in kidney. Diabetic nephropathy is an increasing comorbidity of diabetes. Here, elevation of serum creatinine in diabetic rats was blunted by *P. soyauxii* treatment but not by GB. Consistently, AE of *P. soyauxii*-treated but not GB-treated rats had reduced BW loss (muscle atrophy). The observation highlights a possible nephroprotective potential of the plant.

Elevation of liver transaminases is a common feature of diabetes directly related to increased amino acids availability.[16] Here, STZ-diabetic rats had increased ALT activity in serum while AST elevation was not significant. Both AE and OE of *P. soyauxii* significantly decreased the ALT/AST transaminases. This indicates liver protection, by possibly restored insulin secretion, as described with *P. marsupium* bark extracts.[16]

Elevation of total cholesterol and triglycerides is a hallmark of metabolic disorders often linked to diabetes, as a consequence of impaired insulin secretion or insulinoresistance and subsequent increased mobilization of free fatty acids.[20] The AE and OE of *P. soyauxii* normalized serum triglycerides and total cholesterol after 2 weeks treatment in STZ-diabetic rats. This *P. soyauxii* impact contrasted with the lack of protective effect of GB and is consistent with the better antihyperglycemic effect of the plant. Similar antihyperlipidemic effects have been reported with ethanol bark extracts of *P. santalinus*[20] and methanol and aqueous bark extracts of *P. marsupium* Roxb,[16] the effect being attributed to a flavonoid (pterosupin).[4] Antihyperlipidemic activity of *P. soyauxii* extracts may have cardiovascular protective impact in diabetes.

Both aqueous and CH_2Cl_2/CH_3OH extracts of *P. soyauxii* demonstrated blood glucose lowering efficacy. This was achieved in acute administration with high doses (AE 300 mg/kg and OE 150 mg/kg) while in prolonged administration lower doses (AE 150 and OE 75 mg/kg) were efficient. The better efficiency of the OE compared to AE low doses suggests that the active compounds should be more liposoluble than hydrosoluble. The bad OE dose-response, with reduced efficiency of the high doses, could be due to glycosides or antagonist compounds in OE extracts. Conversely, the clear AE dose response, with performing antihyperglycemic high doses, suggests that adverse compounds were not or slowly water extracted.

The present preliminary phytochemical analyses of aqueous AE and organic OE *P. soyauxii* stem barks extracts is consistent with previous reports.[19] Recently, three new benzofurans (1–3) and one new isoflavan (4), pteroyanin G, H, I, and J, together with 21 known compounds, were isolated from the heartwood of *P. soyauxii*.[27] Other species of the *Pterocarpus* genus (*P. marsupium*[4] and *P. santalinus*[18]) present similarities in phytochemical composition with *P. soyauxii* and also contain other phenolic constituents.[28] Most compounds evidenced in the present *P. soyauxii* Taub bark extracts could be involved in antidiabetic activity, in particular flavonoids, tannins,[4] terpenes,[11] saponins,[7] and phenolic compounds.[9]

Phytocompounds exert antidiabetic action by stimulating insulin secretion (pterostilbene, Roxb-(-)-epicatechin)[28] and β cells regeneration (flavonoid),[4] improving insulin sensitivity or insulin-like effect (vestitol, claussequinone),[29] activating liver peroxisome proliferator-activated receptor (pterostilbene), modifying glucose intestinal or hepatic metabolism.[20] In addition, *P. marsupium* extracts may have potent dipeptidyl-peptidase-4 inhibitory action and hypoglycemic action by increased plasma active glucagon-like peptide-1 levels.[30] Further pharmacological, biological, and biochemical investigations will be required to clarify the mechanism of action and the main active principles of *P. soyauxii* Taub stem bark responsible for the antidiabetic effects. In particular, measurement of blood insulin, glucose tolerance tests, evaluation of pancreatic β cells destruction/regeneration, and investigation of skeletal muscle glucose metabolism will bring information. In addition, translation to other animal models will be necessary to test extension of the antihyperglycemic effect to other Type 1 and Type 2 diabetic models. Safety of oral *P. soyauxii* utilization in rodents has been previously shown.[19] The present demonstration of an antidiabetic effect of *P. soyauxii* in STZ-diabetic rats is a further valuable step toward *P. soyauxii* Taub as antidiabetic therapy which could be relevant to human pathology.

CONCLUSION

The study shows that aqueous and CH_2Cl_2/CH_3OH stem bark extracts of *P. soyauxii* Taub have only moderate hypoglycemic impact and potent antihyperglycemic effects with improved metabolic parameters in experimental STZ-diabetic rats, compared to GB. Therefore, *P. soyauxii* extracts may be useful for alternative oral treatment of diabetes and related metabolic disorders. Further studies are needed to address the mechanism of action for *P. soyauxii* in treating diabetes.

Financial support and sponsorship

This work was funded by the Third World Academy of Sciences (Grant 06-020RG/BIO/AF/AC-UNESCOFR 3240144852), the French-Cameroon Cooperation (French Embassy in Cameroon) and INSERM, U1048,-I2MC, Toulouse, France.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas Update Poster. 6th ed. Brussels (Belgium): International Diabetes Federation; 2014.
2. Peer N, Kengne AP, Motala AA, Mbanya JC. Diabetes in the Africa Region: An update. Diabetes Res Clin Pract 2014;103:197-205.
3. Mbanya JC, Motala AA, Sobngwi E, Assah FK, Enoru ST. Diabetes in sub-Saharan Africa. Lancet 2010;375:2254-66.
4. Rizvi SI, Mishra N. Traditional Indian medicines used for the management of diabetes mellitus. J Diabetes Res 2013;2013:712092.

5. CDiC Project (Changing Diabetes in Children). "Changing diabetes in children." Cameroon: CDIC Project Management Manual, Cameroon/Region; 2010. p. 44.
6. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012;2:320-30.
7. Elekofehinti OO. Saponins: Anti-diabetic principles from medicinal plants – A review. Pathophysiology 2015;22:95-103.
8. Hung HY, Qian K, Morris-Natschke SL, Hsu CS, Lee KH. Recent discovery of plant-derived anti-diabetic natural products. Nat Prod Rep 2012;29:580-606.
9. Vinayagam R, Jayachandran M, Xu B. Antidiabetic effects of simple phenolic acids: A comprehensive review. Phytother Res 2016;30:184-99.
10. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, *et al*. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 2015;20:21138-56.
11. Mohammed A, Ibrahim MA, Islam MS. African medicinal plants with antidiabetic potentials: A review. Planta Med 2014;80:354-77.
12. Kibiti CM, Afolayan AJ. Herbal therapy: A review of emerging pharmacological tools in the management of diabetes mellitus in Africa. Pharmacogn Mag 2015;11 Suppl 2:S258-74.
13. Kuete V, Efferth T. Cameroonian medicinal plants: Pharmacology and derived natural products. Front Pharmacol 2010;1:123.
14. Ntie-Kang F, Lifongo LL, Mbaze LM, Ekwelle N, Owono Owono LC, Megnassan E, *et al*. Cameroonian medicinal plants: A bioactivity versus ethnobotanical survey and chemotaxonomic classification. BMC Complement Altern Med 2013;13:147.
15. Saliu JA, Elekofehinti OO, Komolafe K, Oboh G. Effects of some green leafy vegetables on the haematological parameters of diabetic rats. J Nat Prod Plant Resour 2012;2:482-5.
16. Dhanabal SP, Kokate CK, Ramanathan M, Kumar EP, Suresh B. Hypoglycaemic activity of *Pterocarpus marsupium* Roxb. Phytother Res 2006;20:4-8.
17. Kameswara Rao B, Giri R, Kesavulu MM, Apparao C. Effect of oral administration of bark extracts of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. J Ethnopharmacol 2001;74:69-74.
18. Bulle S, Reddyvari H, Nallanchakravarthula V, Vaddi DR. Therapeutic potential of *Pterocarpus santalinus* L.: An update. Pharmacogn Rev 2016;10:43-9.
19. Tchamadeu MC, Dzeufiet PD, Nana P, Kouambou Nouga CC, Ngueguim Tsofack F, Allard J, *et al*. Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of *Pterocarpus soyauxii* Taub (*Papilionaceae*) in rodents. J Ethnopharmacol 2011;133:329-35.
20. Kondeti VK, Badri KR, Maddirala DR, Thur SK, Fatima SS, Kasetti RB, *et al*. Effect of *Pterocarpus santalinus* bark, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol 2010;48:1281-7.
21. Tchamadeu MC, Dzeufiet PD, Nouga CC, Azebaze AG, Allard J, Girolami JP, *et al*. Hypoglycaemic effects of *Mammea africana* (Guttiferae) in diabetic rats. J Ethnopharmacol 2010;127:368-72.
22. Sakata N, Yoshimatsu G, Tsuchiya H, Egawa S, Unno M. Animal models of diabetes mellitus for islet transplantation. Exp Diabetes Res 2012;2012:256707.
23. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, *et al*. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21:15-23.
24. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010;1:87-93.
25. Fröde TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 2008;115:173-83.
26. Vats V, Grover JK, Rathi SS. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. J Ethnopharmacol 2002;79:95-100.
27. Su Z, Wang P, Yuan W, Li S. Chemical constituents from *Pterocarpus soyauxii*. Nat Prod Commun 2014;9:1483-6.
28. Mishra A, Srivastava R, Srivastava SP, Gautam S, Tamrakar AK, Maurya R, *et al*. Antidiabetic activity of heart wood of *Pterocarpus marsupium* Roxb. and analysis of phytoconstituents. Indian J Exp Biol 2013;51:363-74.
29. Mohankumar SK, O'Shea T, McFarlane JR. Insulinotrophic and insulin-like effects of a high molecular weight aqueous extract of *Pterocarpus marsupium* Roxb. hardwood. J Ethnopharmacol 2012;141:72-9.
30. Kosaraju J, Dubala A, Chinni S, Khatwal RB, Satish Kumar MN, Basavan D. A molecular connection of *Pterocarpus marsupium*, *Eugenia jambolana* and *Gymnema sylvestre* with dipeptidyl peptidase-4 in the treatment of diabetes. Pharm Biol 2014;52:268-71.

Marie Claire Tchamadeu

ABOUT AUTHOR

Marie Claire Tchamadeu, Department of Animal Biology Organisms, Faculty of Science, University of Douala, P.O. Box: 24157, Douala, Cameroon. E-mail: marieclaire_tchamadeu@yahoo.fr